ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 729–732
0066-4804/86/110729-04$02.00/0

Vol. 30, No. 5

Extravascular Penetration of Highly Protein-Bound Flucloxacillin

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Received 5 February 1986/Accepted 15 August 1986

The pharmacokinetics of intravenously administered flucloxacillin (2.0 g to five volunteers) are described. The passage of flucloxacillin to peripheral lymph and suction skin blisters was monitored. This drug was selected because the high serum protein binding of 96% offered a particularly good opportunity for the study of the impact on tissue penetration. Flucloxacillin was assayed by high-pressure liquid chromatography, and pharmacokinetics were assayed by computerized curve fitting to accepted models. Penetration of flucloxacillin to extravascular foci was rapid. After 30 min the drug concentrations were 0.5 ± 0.3 μg/ml in lymph and 0.9 ± 0.7 μg/ml in blister fluid. The peak concentration was 11.7 ± 5.6 μg/ml in lymph and 4.6 ± 1.4 μg/ml in blister fluid. Concentrations in urine were above 111 ± 50 μg/ml throughout the 8-h monitoring period, and urinary recovery was 60.4%. The half-life was 2.1 ± 0.9 h in serum, 1.4 ± 0.6 h in lymph, and 11.0 ± 4.1 h in blister fluid. The differences in half-life were significant ($P < 0.05$) between serum and blister fluid but not between lymph and serum. Penetration, as represented by the mean ratios of areas under the curve, was 19.7 ± 8.1% to lymph and 38.2 ± 11.7% to blister fluid. The flucloxacillin distribution volume during the phase of elimination was 36.4 ± 16.0 liters and the total body clearance was 12.9 ± 5.5 liters. Flucloxacillin showed good tissue penetration, considering its very high serum protein binding. High flucloxacillin levels in lymph and blister fluid were explained in part by drug affinity to extravascular albumin. The major impacts of high protein binding are (i) slightly slower passage into extravascular sites, (ii) slightly later peak concentration, and (iii) levels in extravascular fluid that are persistently below those in serum.

Very high serum protein binding reduces the fraction of molecules which can freely pass the vascular lining into tissues. We have studied the penetration into peripheral human lymph of a series of antibacterial agents with serum protein binding ranging from 0% (gentamicin) (3) to 85% (temocillin) (4). A distinct but not dramatic impact of the role of protein binding in reducing the extravascular penetration has been observed. Gentamicin shows overlapping levels in lymph and serum (3). Mecillinam with a 5% protein binding reaches lymph concentrations which are 97% of the levels in serum (7). The lymph concentrations of temocillin are 60% of the levels in serum (4). The last observation raises the question of whether a very high serum protein binding (above 80%) necessarily reduces extravascular penetration, as has been theoretically supported (8) and documented with cantharidine skin blisters (9).

In this study we were concerned with the penetration of highly bound flucloxacillin, with 96% protein binding (1), to extravascular sites like suction skin blisters and peripheral lymph in human volunteers.

MATERIALS AND METHODS

Subjects. Five healthy volunteers (one female and four males, ages 25.3 ± 3.0 years [range, 20 to 28 years]; height, 177.6 ± 5.4 cm [range, 171 to 183 cm]; weight, 71.8 ± 6.1 kg [range, 65 to 80 kg]) participated in this study. They were healthy as evidenced by clinical examination and the following laboratory tests: hemoglobin, hematocrit, erythrocyte and leukocyte count, erythrocyte sedimentation rate, serum creatinine, serum bilirubin, alanine aminotransferase (ALAT; serum glutamic pyruvic transaminase), aspartate aminotransferase (ASAT; serum glutamic oxalacetic transaminase), lactate dehydrogenase, alkaline transaminases, Na, K, total albumin, urea, and urinalysis. Lack of penicillin allergy was ascertained by past history and a skin provocation test with flucloxacillin.

The volunteers were provided with oral and written information on the substance, the possible side effects, and the procedures of the study. Written consent was obtained. Insurance covered the study. The volunteers were free to withdraw from the study at any time. The study was carried out in accordance with the 1975 Tokyo revision of the Helsinki Declaration on Studies in Human Subjects. The study was accepted both by the Ethical Committee of Radiumhospitalet and the Norwegian Board of Drug Licensing (Statens Legemiddelkontroll, Oslo, Norway).

Antibiotic. Flucloxacillin for intravenous administration (3-min bolus injection) of 2.0 g were obtained in vials (Beecham Pharmaceuticals, Betchworth, Surrey, United Kingdom) which were reconstituted to a 10% solution immediately prior to administration.

Sampling. Blood samples were drawn from the cubital vein (contralateral to the intravenous infusion site) at 0, 5, 10, 15, 20, 30, and 45 min and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after drug administration. Serum was separated within 45 min. Lymph was collected through a capillary Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) catheter inserted directly into a major subcutaneous collecting lymph vessel as described previously (10). Skin blisters were produced by application of suction to the skin surface by a previously established procedure (11). Each blister was

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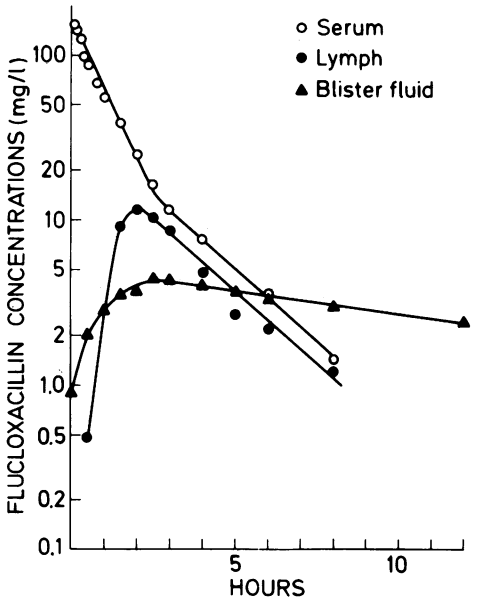

FIG. 1. Mean concentrations of flucloxacillin in serum, peripheral lymph, and suction skin blister fluid of five healthy volunteers who received 2.0 g of drug intravenously.

sampled only once; 0.1 to 0.15 ml of fluid was taken. Lymph and blister fluid were drawn after 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 h. Urine was collected during the intervals 0 to 2, 2 to 4, 4 to 6, and 8 to 12 h. Samples were stored at −70°C until assay.

Assay. Flucloxacillin quantitation was done by high-pressure liquid chromatography (HPLC). Serum proteins were separated by acidification with 100 μl of 2 N HCl and 4 ml of dichloromethane, followed by agitation with a Whirlimixer for 3 min and centrifugation at 6,000 × *g*. Lymph and blister fluid samples were treated analogously. The aqueous supernatant was removed, and the lower organic layer was transferred to a clean tube followed by desiccation under a stream of N_2 and reconstitution to 150 μl by the HPLC eluant before injection onto the HPLC column. Urine samples were diluted 110 times in 0.04 sodium phosphate buffer (pH 7.0) before injection. Standards covering the range of concentrations observed were prepared daily in the respective fluids, and cloxacillin (Astra Läkemedel, Södertälje, Sweden) was used as the internal standard.

HPLC was carried out on a liquid chromatograph (LC4; The Perkin-Elmer Corp., Norwalk, Conn.) equipped with an Autosampler 1SS-100 (Perkin-Elmer), a Spectrophotometer II detector (Laboratory Data Control) and a laboratory computing integrator (LCI-100; Perkin-Elmer). The reverse phase was 25-cm Spherisorb octadecylsilane, with a particle size of 5 μm in diameter (Bischoff, Leonberg, Federal Republic of Germany) with an attached guard column (7.5-cm Spherisorb octadecylsilane; particle size, 5 μm in diameter). The columns were obtained from Crompack (Middelburg, The Netherlands).

The mobile phase was 45% methanol in 0.04 M sodium phosphate buffer (pH 7.0) with a flow rate of 1 ml/min. A sample volume of 10 μl was injected onto the column. The detector light wavelength was 220 nm.

The lower limit of detection was 0.2 μg/ml, and the coefficient of variation was 2.7% at 2 μg/ml and 5.3% at 10 μg/ml.

Pharmacokinetics. Pharmacokinetic assessment was done by AUTOAN 2/NONLIN, and equations have been described elsewhere (12). The first-order one-compartment open model was applied to the blister fluid and lymph data, and the curves for serum were fitted to the two-compartment model.

Statistical analysis. The standard deviation and the Wilcoxon nonparametric test for assessment of significance of differences were applied (13).

TABLE 1. Peak levels of flucloxacillin in serum, peripheral lymph, and suction skin blisters in five healthy volunteers after a single intravenous dose of 2.0 g[a]

Body fluid	C_{max} (μg/ml)	T_{max} (h)
Serum	154.6 ± 60.7	NA[b]
Lymph	11.7 ± 5.6	1.8 ± 0.3
Blister fluid	4.6 ± 1.4	3.4 ± 1.1

[a] Mean concentrations (C_{max}) ± standard deviation and time of occurrence (T_{max}) ± standard deviation are given.
[b] NA, Not applicable.

RESULTS

Serum, lymph, and blisters. Extravascular concentrations of drug were detected soon after drug administration and were 0.5 ± 0.3 μg/ml in lymph and 0.9 ± 0.7 μg/ml in blister fluid, even in the first samples. The mean individual peak concentration was 11.7 ± 5.6 μg/ml in lymph (reached after 1.8 ± 0.3 h) and 4.6 ± 1.4 μg/ml in blister fluid (reached after 3.4 ± 1.1 h). Immediately after the end of infusion, the concentration in serum was 154 ± 60.7 μg/ml. The mean curve of the peripheral concentration in lymph was parallel to the curve for serum, whereas blister fluid concentrations showed slower disposition (Fig. 1). The alpha-phase of rapid disposition of serum lasted for a relatively long time, up until 3 h after drug administration. This corresponded approximately to the point in time when the peaks occurred in extravascular foci.

The ratio of the extravascular peaks and the concentration in serum immediately after infusion was stopped was 49.9 ± 30.9% for lymph and 55.2 ± 33.1% for blister fluid (Table 1). The relatively high level in blister fluid toward the end of the observation period is a function of the slower disposition from blisters.

Urine. The lowest concentration of flucloxacillin in urine during the 8-h monitoring period was 111 ± 50 μg/ml (Table 2). Elimination of flucloxacillin in urine was nearly completed after 6 h and reached 60.4% of the dose.

Pharmacokinetics. The half-life was 2.1 ± 0.9 h for serum, 1.4 ± 0.6 h for lymph, and 11.0 ± 4.1 h for blister fluid (Table 3). The rate of penetration into lymph was faster than that into blisters ($P < 0.05$). The rate of elimination from blisters was slower than that from lymph or serum. Extravascular penetration reflected by the ratio of areas under the concentration curves to infinity was 19.7 ± 8.1% for lymph and 38.2 ± 11.7% for blister fluid.

TABLE 2. Elimination of flucloxacillin in urine in five healthy volunteers after a single intravenous dose of 2.0 g[a]

Collection period (h)	Concn (μg/ml)	Cumulative recovery (%)
0 to 2	8,240 ± 2,920	51.0 ± 9.0
2 to 4	1,930 ± 980	58.4 ± 9.4
4 to 6	290 ± 80	60.0 ± 9.9
6 to 8	110 ± 50	60.4 ± 9.8

[a] Values are means ± standard deviations.

TABLE 3. Pharmacokinetic parameters[a] of flucloxacillin in serum, peripheral lymph, and suction skin blisters in five healthy volunteers after a single intravenous dose of 2.0 g

Body fluid	Lag period (h)	k_1 (h^{-1})	k_E (h^{-1})	C_0 (μg/ml)	$AUC_{0-\infty}$ (μg · h/ml)	$t_{1/2}$ (h)	Ratio of AUC for lymph/blister:serum (%)
Serum	NA[b]	NA	1.139 ± 0.604	182.6 ± 71.9	178.6 ± 69.4	2.1 ± 0.9	
Lymph	0.9 ± 0.1	2.301 ± 1.129	0.502 ± 0.166	20.84 ± 10.83	35.3 ± 20.6	1.4 ± 0.6	19.7 ± 8.1
Blister fluid	0.5 ± 0.1	0.567 ± 0.782	0.071 ± 0.025	5.4 ± 1.7	74.1 ± 25.4	11.0 ± 4.1	38.2 ± 11.7

[a] Pharmacokinetic parameters are as follows: k_1, rate of penetration; k_E, rate of elimination; C_0, initial concentration; AUC, area under the concentration curve to infinity; $t_{1/2}$, half-life. Values are means ± standard deviations.
[b] NA, Not applicable.

TABLE 4. Pharmacokinetic parameters[a] of flucloxacillin in five healthy volunteers after a single intravenous dose of 2.0 g

k_{21} (h^{-1})	k_{12} (h^{-1})	V_1 (liter)	V_{ss} (liter)	V_{area} (liter)	CL_B (liter/h)
0.666 ± 0.413	0.550 ± 0.554	12.2 ± 4.2	20.6 ± 6.5	36.0 ± 16.0	12.9 ± 5.4

[a] Pharmacokinetic parameters are as follows: k_{21}, rate of transfer from compartment 2 to compartment 1; k_{12}, rate of transfer from compartment 1 to compartment 2; V_1, volume of distribution of the central compartment; V_{ss}, volume of distribution at steady state; V_{area}, distribution volume; CL_B, body clearance. Values are means ± standard deviations.

The distribution volume of flucloxacillin was 36.4 ± 16.0 liters, and total body clearance was 12.9 ± 5.5 liters/h (Table 4).

DISCUSSION

Flucloxacillin showed a fairly good tissue penetration, as reflected by the concentration in peripheral lymph and suction skin blisters. This occurred in spite of the very high serum protein binding of 96%, leaving only 4% as free drug readily available for passage across the vascular lining. Concentrations of flucloxacillin in lymph were 20% of those in serum. This penetration ratio compares with 97% for mecillinam, which has a protein binding of 5% (7); a penetration ratio of 78 to 88% for ampicillin and amoxicillin, which have protein bindings of 15% (6); a penetration ratio of 64 to 71% for two doses of the ureidopenicillin BRL 36650, which has a protein binding of 30% (T. Bergan, A. Engeset, W. Olszewski, and N. Larson, manuscript in preparation); and a penetration ratio of 58% for temocillin, which has a protein binding of 85% (4). The penetration of flucloxacillin thus corresponds quite well to what one can extrapolate from penetration ratios of other penicillins relative to their serum protein binding.

Other factors than protein binding also have a significant impact on the levels in lymph compared with those in serum. Penicillins with a half-life below 2 h disappear slightly more slowly from lymph than from serum; accordingly, concentrations in lymph are above those in serum toward the end of normal dosing intervals in such cases. A long serum half-life, as shown by temocillin and its relatively high protein binding, explains why temocillin shows parallel curves for concentrations in serum and extravascular fluid and concentrations in serum above those in lymph. Penicillins are only bound to albumin and show a relatively fast elimination rate from the extravascular foci compared with that of erythromycin, which is retained within the extravascular space by the fixed lattice of alpha-2-globulin fibers and shows a lymph curve course that is more horizontal (5).

The unusually high serum protein binding of flucloxacillin is balanced by binding to extravascular albumin. Once a free molecule has passed the vascular lining, it may become attached to extravascular albumin and thus enter an extravascular pool of molecules that act as ligands. This explains why even antibiotics with high serum protein binding may reach high extravascular levels and a penetration ratio exceeding that of the unbound moiety in serum. The percentage of protein binding in lymph is unknown, but the concentrations of albumin in lymph are 60 to 70% of those in serum (2). The distribution of flucloxacillin compared well with those found for aminopenicillins and mecillinam, which have minimal protein binding; this indicates that protein binding has little impact on distribution.

In this study we compared the extravascular concentration of flucloxacillin in different extravascular fluids, represented by peripheral lymph and blisters. Although drug concentrations in both fluids were in balance with that in serum, the interplay between serum and lymph is more immediate than blister fluid, which has a larger void volume; lymph represents continuous drainage from the interstitial space. The levels in peripheral lymph represent the situation in unmanipulated tissues around a focus of infection more than blisters, which resembles the situation more within an infected tissue site, e.g., one with necrosis or small abscesses. The drug level in blisters is relevant for flucloxacillin, considering that its target organisms, staphylococci, are prone to produce abscesses. The results suggest that beta-lactamase-producing staphylococci would be inhibited by flucloxacillin within 30 min of dosing. Peak concentrations in both extravascular fluids were considerably above the MICs for most staphylococci (except methicillin-resistant strains).

We thus conclude that even extremely high serum protein binding does not seem to inhibit tissue penetration seriously. The major impacts of a high protein binding are (i) slightly slower passage into extravascular sites, (ii) slightly later peak concentrations, and (iii) levels in extravascular fluid that are persistently below those in serum.

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